



SEQ_LISTING_11302009
SEQUENCE LISTING

<110> Korea Research Institute of Bioscience and Biotechnology

<120> Method for screening of a lipase having improved enzymatic activity using yeast surface display vector and the lipase

<130> 26666U

<140> 10/527,438

<141> 2005-03-11

<150> PCT/KR03/01820

<151> 2003-09-04

<150> KR 2002-55575

<151> 2002-09-13

<160> 19

<170> PatentIn version 3.5

<210> 1

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> CALB primer 1

<400> 1

ggctttcag ccactcctt ggtgaag

27

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<213> Artificial sequence

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<223> CALB primer 2

<400> 2

gcggatccctc aggggggtgac gat

23

<210> 3

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> CALB primer 3

<400> 3

gcggatccgg gggtgacgat gccggag

27

<210> 4

<211> 19

<212> DNA

<213> Artificial sequence

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<220>
 <223> GPD-err primer

 <400> 4
 gcagagctaa ccaataagg 19

<210> 5
 <211> 19
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> T-0 primer

 <400> 5
 tgcagttgaa cacaaccac 19

<210> 6
 <211> 1023
 <212> DNA
 <213> Candida antarctica

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 gatgcggggtc tgacctgcca gggtgcttcg ccattctcg tctccaaacc catccttctc 180
 gtcccccggaa ccggcaccac aggtccacag tcgttcgact cgaactggat cccctctct 240
 ggcgcagctgg gttacacacc ctgctggatc tcaccccgcc cgttcatgct caacgacacc 300
 caggtcaaca cggagtacat ggtcaacgcc atcaccacgc tctacgctgg ttcgggcaac 360
 aacaagcttc cctgtctcac ctggtcccag ggtggtctgg ttgcacagtg gggtctgacc 420
 ttcttccca gtatcaggtc caaggtcgat cgacttatgg ccttgcgcc cgactacaag 480
 ggcaccgtcc tcgccccccc tctcgatgca ctcgcggta gtgcaccctc cgtatggcag 540
 caaaccaccg gttcggcact cactaccgca ctccgaaacg caggtggtct gacccagatc 600
 gtgcccacca ccaacctcta ctcggcgacc gacgagatcg ttcagcctca ggtgtccaac 660
 tcgcccactcg actcatccta cctcttcaac gggagaacg tccaggcaca ggctgtgtgt 720
 gggccgctgt tcgtcatgca ccatgcaggc tcgctcacct cgcaattctc ctacgtcg 780
 ggtcgatccg ccctgcgctc caccacggc caggctcgta gtgcagacta tggcattacc 840
 gactgcaacc ctcttcccgcc caatgatctg actcccggac aaaaggtcgc cgccgctg 900
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<211> 951
<212> DNA
<213> Candida antarctica

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acctgccaag gtgcttcgccc atcctcggtc tccaaaccca tccttctcg t ccccgaaacc 120
ggcaccacag gtccacagtc gttcgactcg aactggatcc ccctctctgc gcagctgggt 180
tacacaccct gctggatctc acccccgcg ttcatgctca acgacacacca ggtcaacacg 240
gagtacatgg tcaacgccc taccacgctc tacgctggtt cgggcaacaa caagcttccc 300
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atcaggtcca aggtcgatcg acttatggcc tttgcgccc actacaaggg caccgtcctc 420
gccggccctc tcgatgcact cgccgttagt gcaccctccg tatggcagca aaccaccgg 480
tcggcactca ctaccgcaact ccgaaacgca ggtggtctga cccagatcg t gcccaccacc 540
aacctctact cggcgaccga cgagatcg t cagcctcagg tgtccaactc gccactcgac 600
tcatcctacc ttttcaacgg aaagaacg t caggcacagg ctgtgtgtgg gccgcagttc 660
gtcatcgacc atgcaggctc gtcaccc t cagttctcct acgtcg t cgg tcgatccgccc 720
ctgcgtc tcca ccacgggcca ggctcgtagt gcggactatg gcattacgga ctgcaaccct 780
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gcccggccct ttgcagtagg caaaaggacc tgctccggca tcgtcacc c 951

<210> 8
<211> 1023
<212> DNA
<213> Candida antarctica

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gatgcggg tgc tgccttcc ggtgc ttcg ccatcctcgg tctccaaacc catccttctc 180
gtccccggaa cccggcaccac aggtccacag t cgttgcact cgaactggat cccctctct 240
gcgcagctgg gttacacacc ctgctggatc tcaccccccgc cgttcatgct caacgacacc 300
cagg tcaaca cggag t acat ggtcaacg cccatcaccacgc tctacgctgg ttcggcaac 360
aacaagcttc c cgtgctc ac tgg tccag ggtgg tctgg ttgcacagtg ggg tctgacc 420
ttcttccca g t a t cagg tgc tcaagg t cgtatgg ccttgc gccc c gactacaag 480
ggcaccgtcc t cgc cggccc tctcgatgca ctcgcgg tta gtcaccctc cgtatggcag 540
caaaccaccg gttcggcact cactaccgca ctccgaaacg cagg tgg tct gacccagatc 600

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gtgcccacca ccaacctcta ctcggcggacc gacgagatcg ttcagcctca ggtgtccaa	660
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ggtcgatccg ccctgcgctc caccacgggc caggctcgta gtgcagacta tggcattacg	840
gactgcaacc ctcttcccgc caatgatctg actcccgagc aaaaggtcgc cgccggctgc	900
ctcctggcgc cggcggctgc agccatcgta gcgggtccaa agcagaactg cgagccgcac	960
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ccc	1023

<210> 9

<211> 319

<212> PRT

<213> Candida antarctica

<400> 9

Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu
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20 25 30

Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe
35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys
50 55 60

Trp Ile Ser Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr
65 70 75 80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn
85 90 95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly
145 150 155 160

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Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His
210 215 220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr
245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val
260 265 270

Ala Ala Ala Ala Leu Pro Ala Pro Ala Ala Ala Ala Ile Val Ala Gly
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro Gly Ser
305 310 315

<210> 10

<211> 319

<212> PRT

<213> Candida antarctica

<400> 10

Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu
1 5 10 15

Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys
20 25 30

Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe
35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys
50 55 60

SEQ_LISTING_11302009

Trp Ile Ser Pro Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr
65 70 75 80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn
85 90 95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly
145 150 155 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Gln Phe Val Ile Asp His
210 215 220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr
245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val
260 265 270

Ala Ala Ala Ala Leu Pro Ala Pro Ala Ala Ala Ile Val Ala Gly
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro Gly Ser
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315

<210> 11
<211> 317
<212> PRT
<213> Candida antarctica

<400> 11

Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu
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Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys
20 25 30

Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe
35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys
50 55 60

Trp Ile Ser Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr
65 70 75 80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn
85 90 95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly
145 150 155 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Gln Phe Val Ile Asp His
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SEQ_LISTING_11302009

210

215

220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala
 225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr
 245 250 255

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val
 260 265 270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly
 275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe
 290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro
 305 310 315

<210> 12
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 <212> DNA
 <213> Artificial Sequence

<220>
 <223> CALB primer 4

<400> 12
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26

<210> 13
 <211> 21
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> a-amylase secretion signal

<400> 13

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Ala Pro Ala Leu Ala
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<210> 14
 <211> 317
 <212> PRT
 <213> Candida antarctica

<400> 14

SEQ_LISTING_11302009

Leu Pro Ser Gly Ser Asp Pro Ala Phe Ser Gln Pro Lys Ser Val Leu
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Asp Ala Gly Leu Thr Cys Gln Gly Ala Ser Pro Ser Ser Val Ser Lys
20 25 30

Pro Ile Leu Leu Val Pro Gly Thr Gly Thr Thr Gly Pro Gln Ser Phe
35 40 45

Asp Ser Asn Trp Ile Pro Leu Ser Ala Gln Leu Gly Tyr Thr Pro Cys
50 55 60

Trp Ile Ser Pro Pro Pro Phe Met Leu Asn Asp Thr Gln Val Asn Thr
65 70 75 80

Glu Tyr Met Val Asn Ala Ile Thr Thr Leu Tyr Ala Gly Ser Gly Asn
85 90 95

Asn Lys Leu Pro Val Leu Thr Trp Ser Gln Gly Gly Leu Val Ala Gln
100 105 110

Trp Gly Leu Thr Phe Phe Pro Ser Ile Arg Ser Lys Val Asp Arg Leu
115 120 125

Met Ala Phe Ala Pro Asp Tyr Lys Gly Thr Val Leu Ala Gly Pro Leu
130 135 140

Asp Ala Leu Ala Val Ser Ala Pro Ser Val Trp Gln Gln Thr Thr Gly
145 150 155 160

Ser Ala Leu Thr Thr Ala Leu Arg Asn Ala Gly Gly Leu Thr Gln Ile
165 170 175

Val Pro Thr Thr Asn Leu Tyr Ser Ala Thr Asp Glu Ile Val Gln Pro
180 185 190

Gln Val Ser Asn Ser Pro Leu Asp Ser Ser Tyr Leu Phe Asn Gly Lys
195 200 205

Asn Val Gln Ala Gln Ala Val Cys Gly Pro Leu Phe Val Ile Asp His
210 215 220

Ala Gly Ser Leu Thr Ser Gln Phe Ser Tyr Val Val Gly Arg Ser Ala
225 230 235 240

Leu Arg Ser Thr Thr Gly Gln Ala Arg Ser Ala Asp Tyr Gly Ile Thr
245 250 255

SEQ_LISTING_11302009

Asp Cys Asn Pro Leu Pro Ala Asn Asp Leu Thr Pro Glu Gln Lys Val
260 265 270

Ala Ala Ala Ala Leu Leu Ala Pro Ala Ala Ala Ala Ile Val Ala Gly
275 280 285

Pro Lys Gln Asn Cys Glu Pro Asp Leu Met Pro Tyr Ala Arg Pro Phe
290 295 300

Ala Val Gly Lys Arg Thr Cys Ser Gly Ile Val Thr Pro
305 310 315

<210> 15

<211> 28

<212> DNA

<213> Artificial sequence

<220>

<223> LQ53 primer

<400> 15

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<210> 16

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> LQ53 primer

<400> 16

gcatggtcga tgacgaactg cggcccacac 30

<210> 17

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> LP53 primer

<400> 17

gtcgccgcgg ctgcgctccc ggcgcccggcg 30

<210> 18

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> LP35 primer

<400> 18

<210> 19
<211> 343
<212> PRT
<213> *Candida antarctica*

<400> 19

Met Asn Ile Phe Tyr Ile Phe Leu Phe Leu Leu Ser Phe Val Gln Gly
1 5 10 15

Thr Ala Thr Pro Leu Val Lys Arg Leu Pro Ser Gly Ser Asp Pro Ala
20 25 30

Phe Ser Gln Pro Lys Ser Val Leu Asp Ala Gly Leu Thr Cys Gln Gly
35 40 45

Ala Ser Pro Ser Ser Val Ser Lys Pro Ile Leu Leu Val Pro Gly Thr
50 55 60

Gly Thr Thr Gly Pro Gln Ser Phe Asp Ser Asn Trp Ile Pro Leu Ser
65 70 75 80

Ala Gln Leu Gly Tyr Thr Pro Cys Trp Ile Ser Pro Pro Pro Phe Met
85 90 95

Leu Asn Asp Thr Gln Val Asn Thr Glu Tyr Met Val Asn Ala Ile Thr
100 105 110

Thr Leu Tyr Ala Gly Ser Gly Asn Asn Lys Leu Pro Val Leu Thr Trp
115 120 125

Ser Gln Gly Gly Leu Val Ala Gln Trp Gly Leu Thr Phe Phe Pro Ser
130 135 140

Ile Arg Ser Lys Val Asp Arg Leu Met Ala Phe Ala Pro Asp Tyr Lys
145 150 155 160

Gly Thr Val Leu Ala Gly Pro Leu Asp Ala Leu Ala Val Ser Ala Pro
165 170 175

Ser Val Trp Gln Gln Thr Thr Gly Ser Ala Leu Thr Thr Ala Leu Arg
180 185 190

Asn Ala Gly Gly Leu Thr Gln Ile Val Pro Thr Thr Asn Leu Tyr Ser
195 200 205

Ala Thr Asp Glu Ile Val Gln Pro Gln Val Ser Asn Ser Pro Leu Asp
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210

215

220

Ser Ser Tyr Leu Phe Asn Gly Lys Asn Val Gln Ala Gln Ala Val Cys
225 230 235 240

Gly Pro Leu Phe Val Ile Asp His Ala Gly Ser Leu Thr Ser Gln Phe
245 250 255

Ser Tyr Val Val Gly Arg Ser Ala Leu Arg Ser Thr Thr Gly Gln Ala
260 265 270

Arg Ser Ala Asp Tyr Gly Ile Thr Asp Cys Asn Pro Leu Pro Ala Asn
275 280 285

Asp Leu Thr Pro Glu Gln Lys Val Ala Ala Ala Leu Leu Ala Pro
290 295 300

Ala Ala Ala Ala Ile Val Ala Gly Pro Lys Gln Asn Cys Glu Pro Asp
305 310 315 320

Leu Met Pro Tyr Ala Arg Pro Phe Ala Val Gly Lys Arg Thr Cys Ser
325 330 335

Gly Ile Val Thr Pro Gly Ser
340